32 0.14 0.12-0.10 0.06 0.04 0.02 60.00 40.00 50.00 70.00 20.00 30.00 10.00 0.00 Minutes

Fig. 2 The HPLC fingerprint of ASHMI at 254nm

FIGURE 2

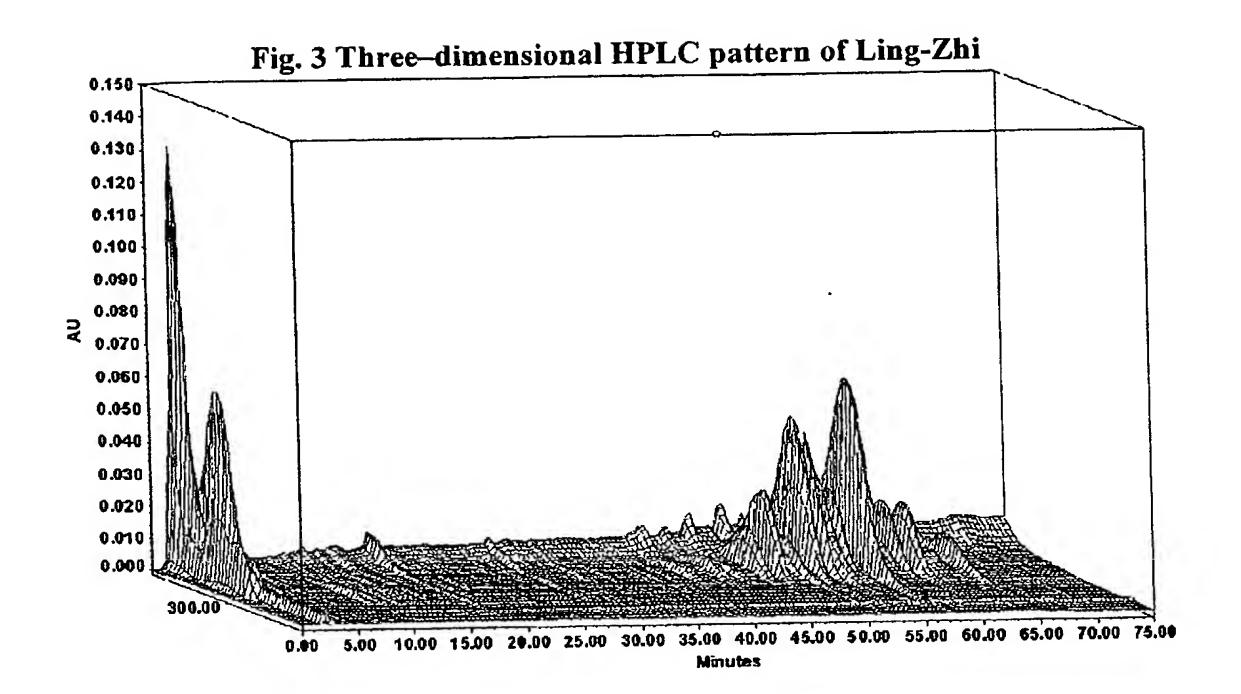


FIGURE 3

Fig 4 The HPLC fingerprint of Ling-Zhi at 254 nm

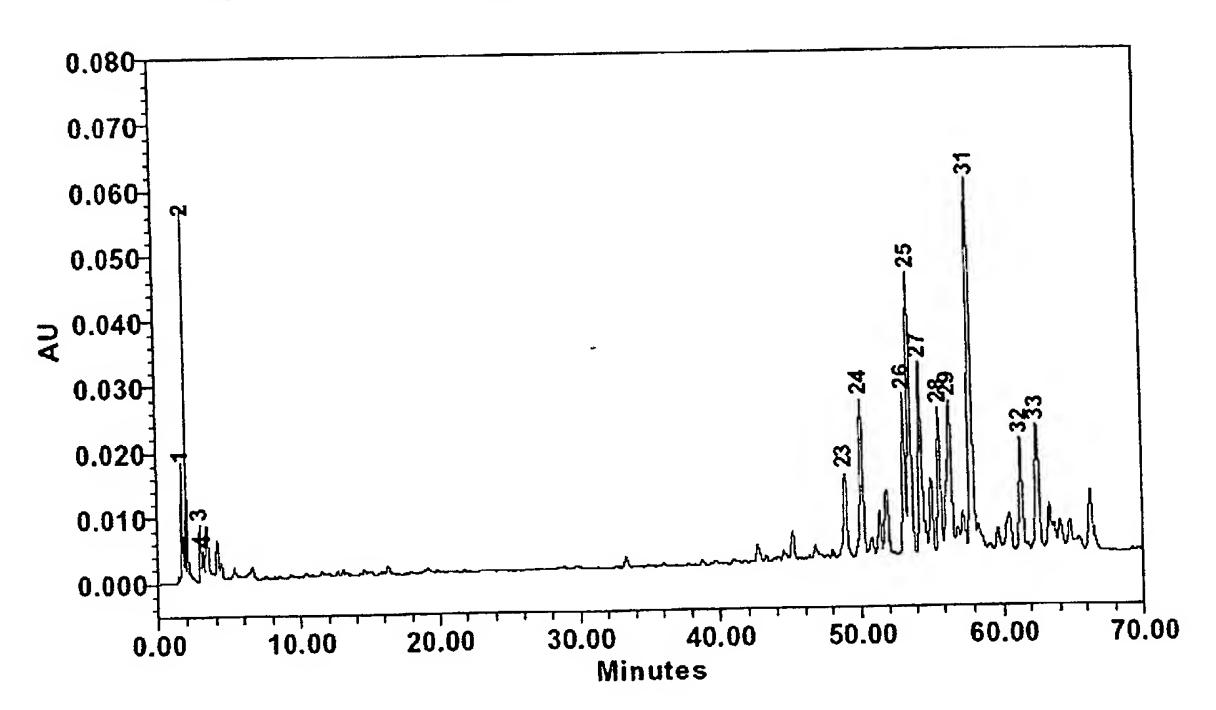


FIGURE 4

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Fig. 5 Three-dimensional HPLC pattern of Ku-Shen

Fig. 6 The HPLC fingerprint of Ku-Shen at 254nm

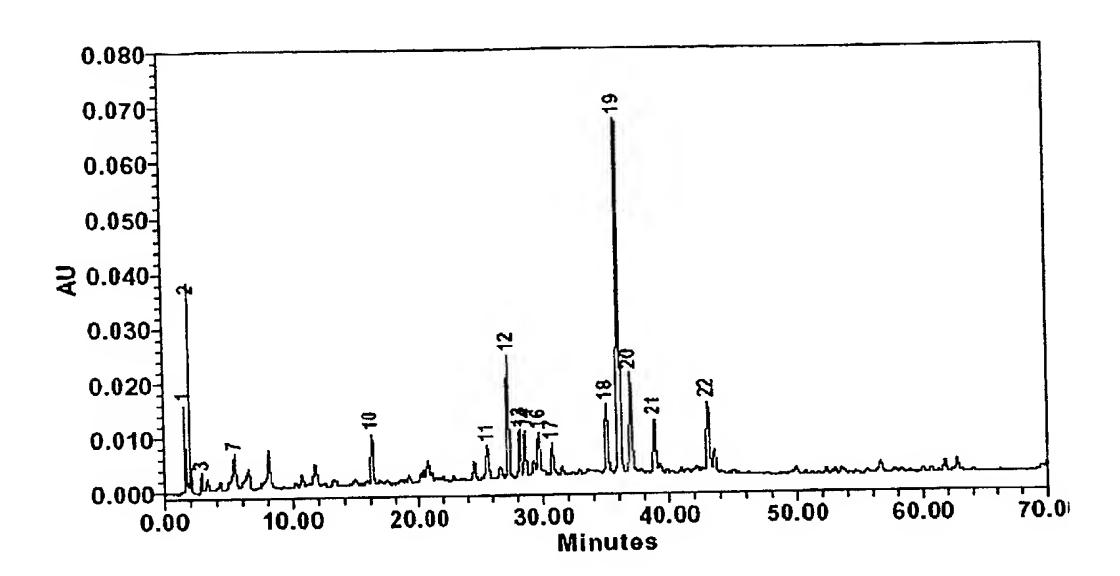


FIGURE 6

0.34 0.32 0.30 0.28 0.26 0.24 0.22 0.20 0.18 0.18 0.14 0.12 0.10 80.0 80.0 0.04 0.02 300.00 5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00 Minutes

Fig.7 Three-dimensional HPLC pattern of Gan-Cao

FIGURE 7

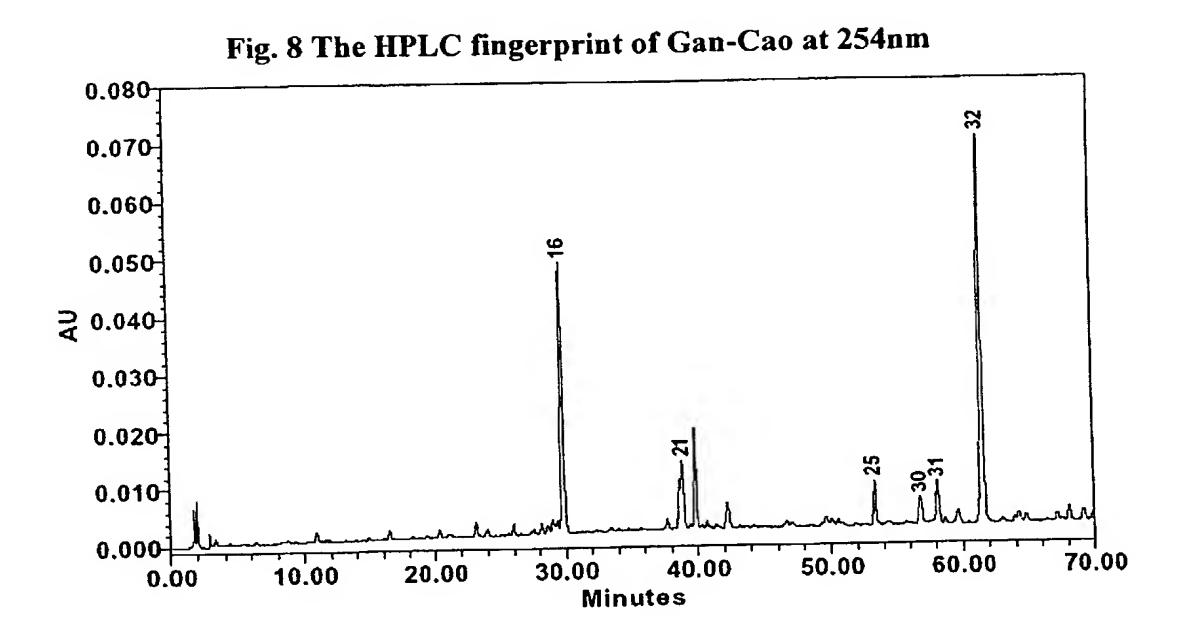


FIGURE 8

Fig. 9 The HPLC profiles of ASHMI and individuals (254nm) 0.50 0.48 Gan-Cao 0.46 0.44 0.42 0.40 ಕ್ಲಿಸ್ಟ 0.38 0.36 0.34 Ku-Shen 0.32 0.30 0.28 ₹ 0.26 0.24 0.22 0.20 Lina-Zhi 0.18 0.16-0.14 0.12 0.10 **ASHMI** 0.08 0.06 0.04 0.02 0.00 0.00 70.00 60.00 20.00 50.00 30.00 40.00 10.00

FIGURE 9

Minutes

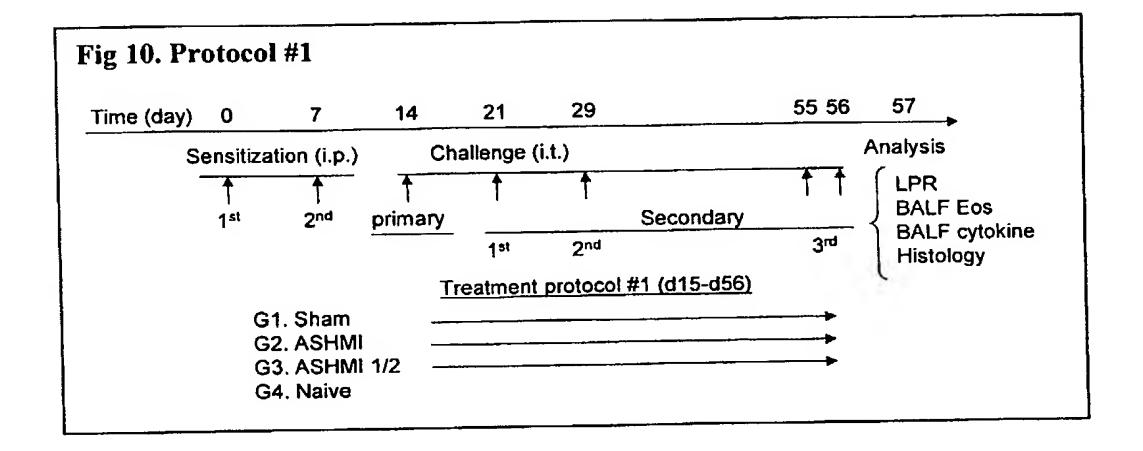


FIGURE 10

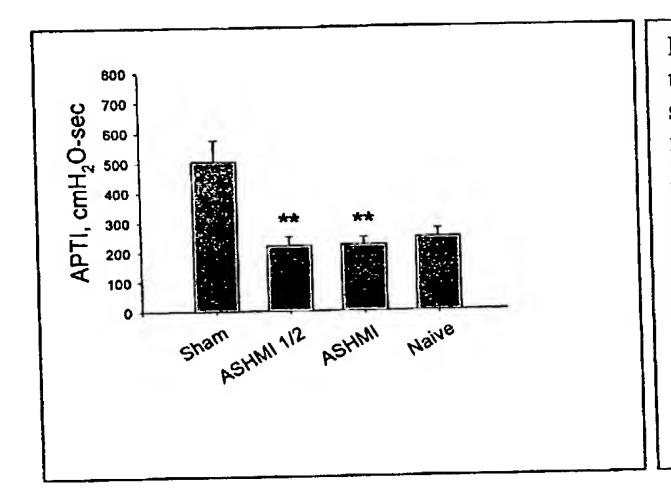


Fig 11. ASHMI
treatment
suppressed Aginduced AHR.

Data are Mean ±
SEM of 6-8 mice
from each
group**p<0.01 vs
sham.

FIGURE 11

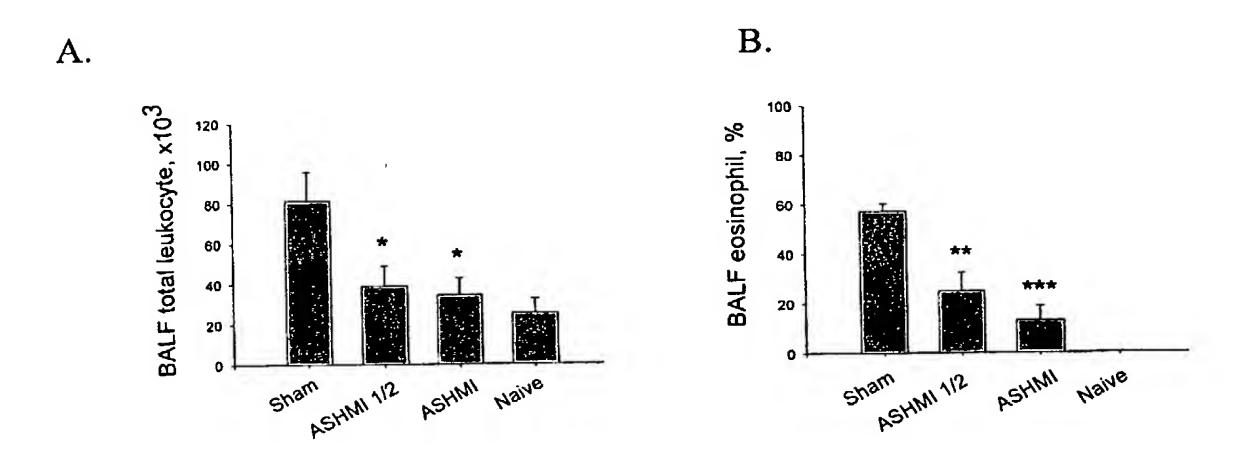


Fig 12. ASHMI reduced Ag-induced pulmonary inflammation: Total number of cells and differential counts of BALF cells were determined by microscopic evaluation. A. shows the total number of cells and B. shows percent of eosinophils. Data are Mean ± SEM of 6-8 mice from each group. *, p<0.05; **, p<0.01 vs sham and ***, p<0.001.

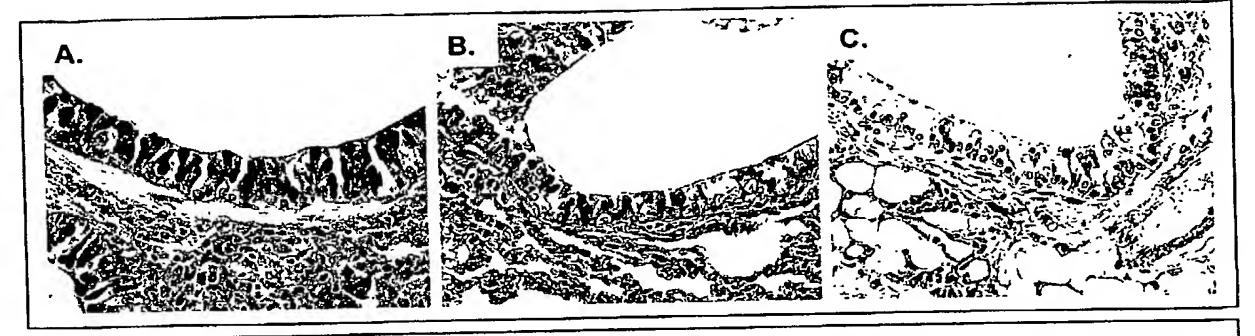


Fig4. Lung histology. Mice in each group (n=4/group) were necropsied after airway response measurement and unlavaged left upper lobe lungs were fixed in neutral buffered formaldehyde. Five-µm paraffin sections were stained with periodic acid-Schiff's reagent (PAS) for goblet cells. A. shows goblet cell hyperplasia in airway from a saline placebo treated mouse. B. illustrates markedly reduced mucus goblet cells in airways of ASHMI treated mice. C. shows absence of goblet cells in airways of naïve mice.

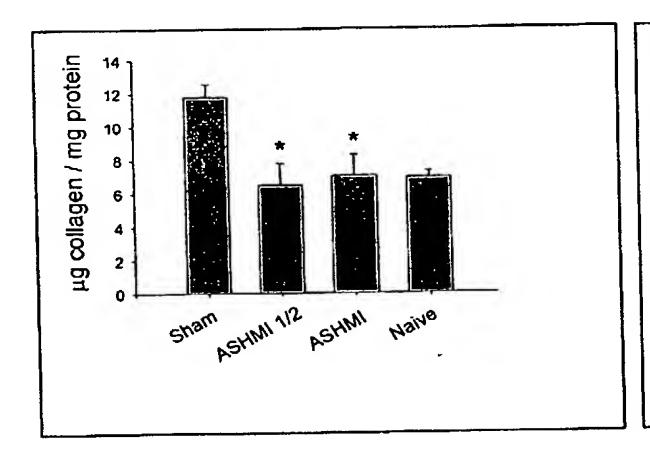


Fig 14.
Decreased
Collagen
content. Data are
Mean ± SEM of
4-5 mice from
each group*,
p<0.05 vs sham.

FIGURE 14

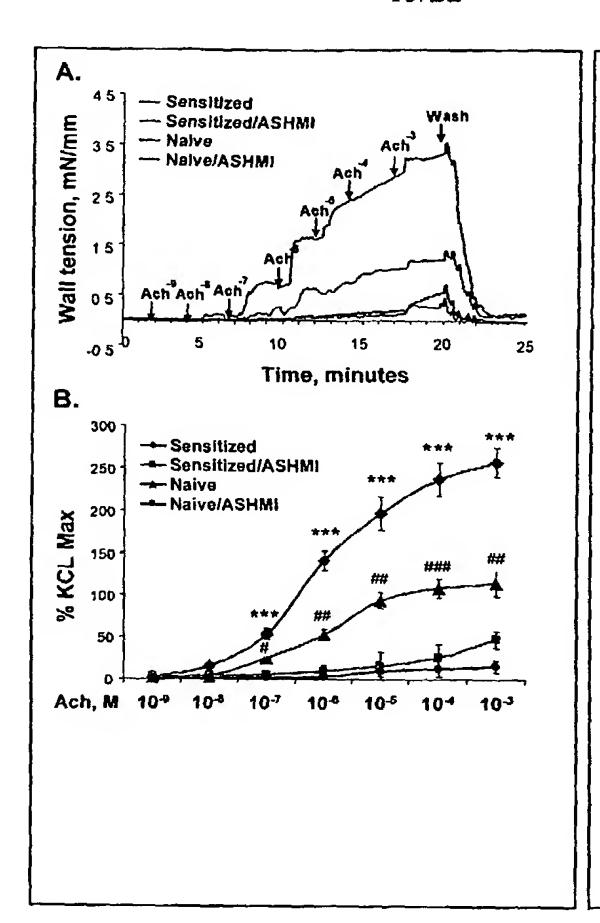
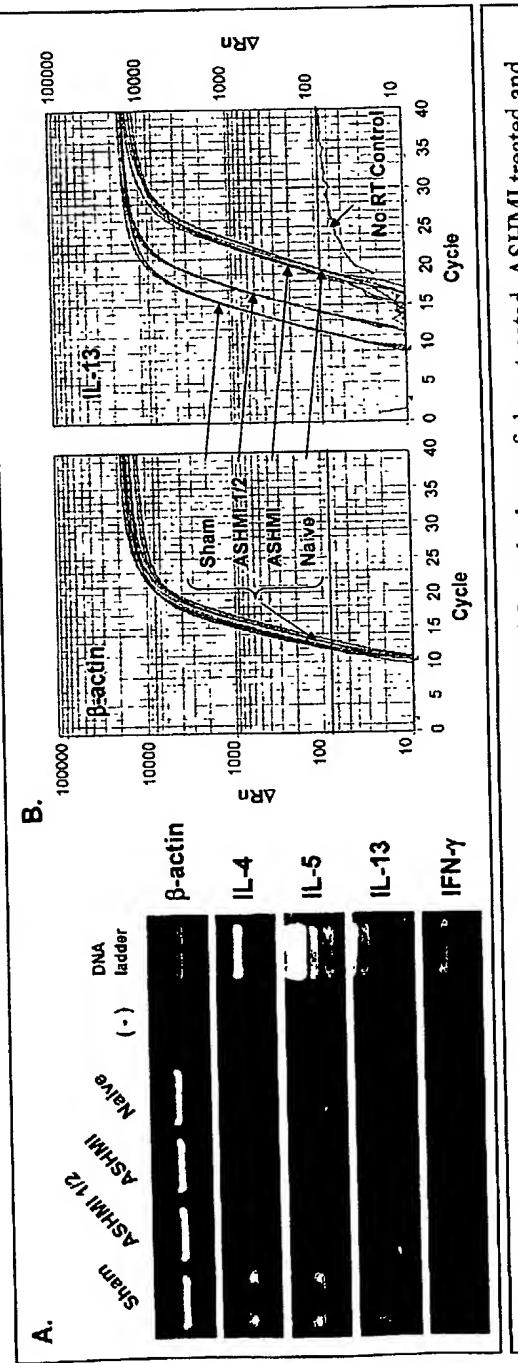


Fig 15. ASHMI inhibits contractile responses of tracheal ring to acetylcholine. Contractile responses to increasing doses of Ach were evaluated in tracheal rings in the presence and absence of 100 ug/ml ASHMI. (A) shows realtime tracing of a typical contractile response. Responses from 4 animals were used to generate a dose response curve to Ach (B). Data are expressed as percent of maximal contraction to 60 mM KCL.**, p<0.01; ***p<0.001 vs, Sensitized/ASHMI. ##; p<0.01; ###, p<0.001vs Naïve/ASHMI.

FIGURE 15



separate RT-PCR experiments from 3 lungs of each group. B. Amplification plots of cDNA samples for IL-13 and β-Fig 16 Semi quantitative RT-PCR. Total RNA was extracted from the lung of sham treated, ASHMI treated and with β -actin. The results are representative of 3 actin. Three cDNA samples from 3 lungs of each group were performed. naïve mice. Gel illustrates cytokine mRNA expression compared

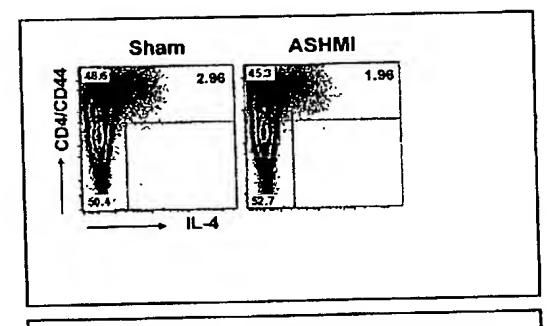
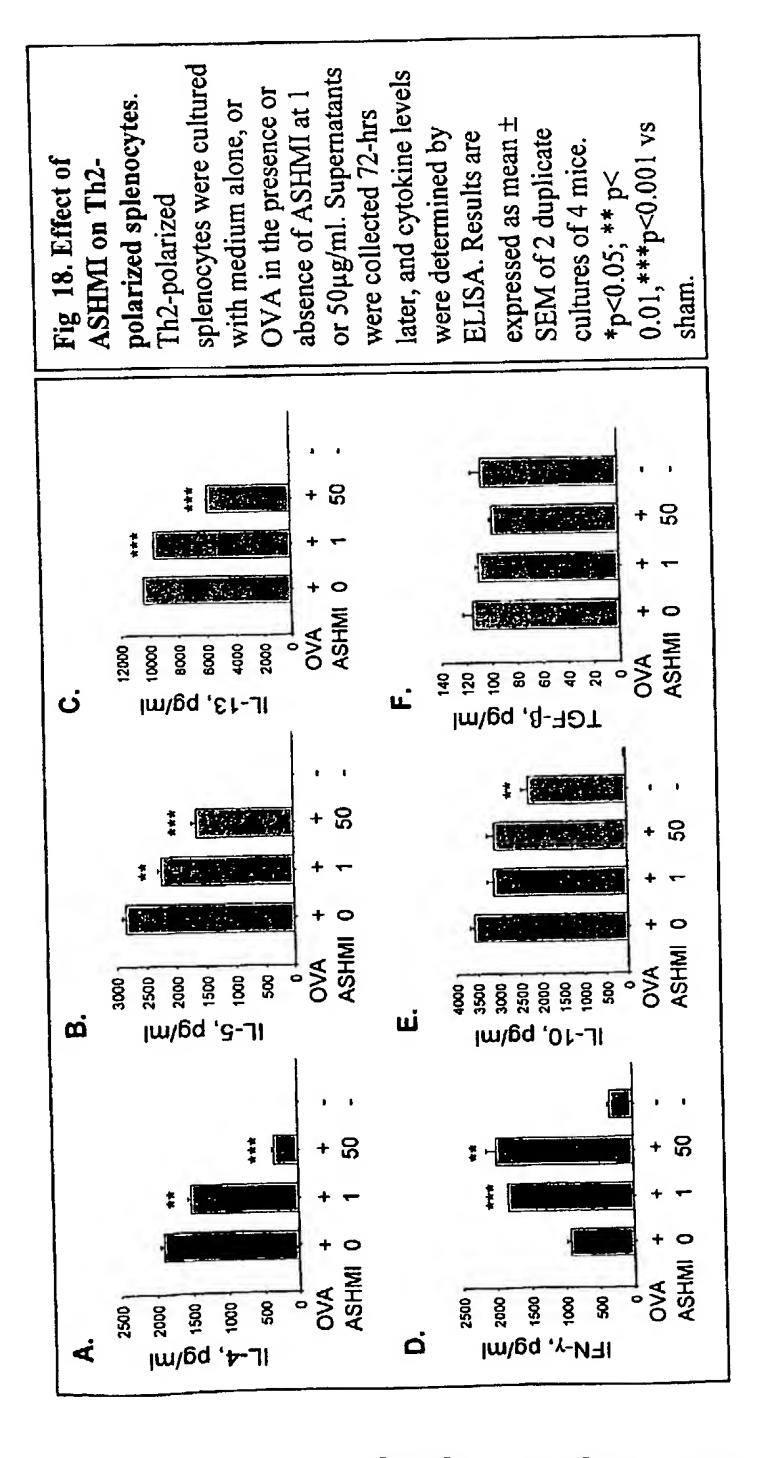


Fig 17. Flow cytometry: Data show the percent of CD4+CD44+ IL-4+ cells.

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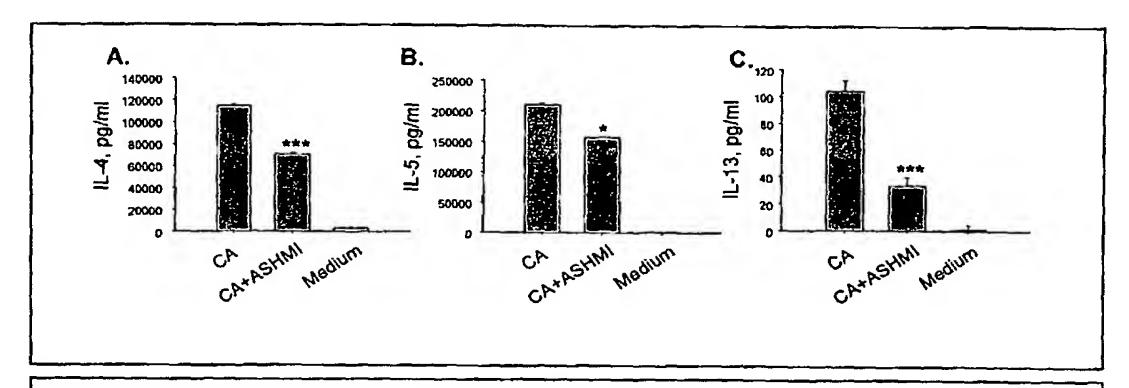


Fig 19. Effect of ASHMI on D10 cells. D10 cells were cultured in the presence of CA and irradiated syngeneic splenocytes in the presence or absence of ASHMI at $50\mu g/ml$. Supernatants were collected 72-hrs later, and cytokine levels were determined by ELISA. Results are expressed as mean \pm SEM of triplicate cultures from three experiments. *p<0.05; ***p<0.001 vs CA.

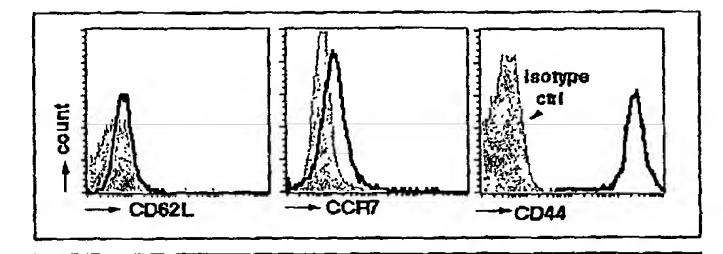


Fig 20. Phenotype of Th2 polarized cell line, D10.G4.1. Flow cytometry results show D10 cells phenotype CD44^{high}CD62L^{neg}CCR7^{neg}(the open histogram).

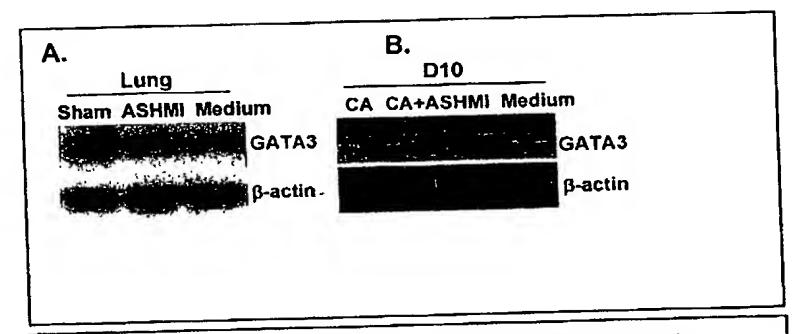


Fig 21. Western blot for determining GATA-3 protein expression in lung tissue (A) and D10 cells (B).

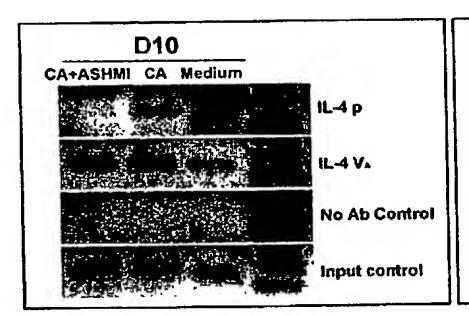


Fig 22. ChIP Assay. Illustration of ChIP assay using a ChIP Assay Kit (Upstate Biotechnology. Lake Placid NY) indicates GATA-3 binding to IL-4 promoter and enhancer in the D10 cells with or without CA-stimulation in the presence or absence of ASHMI treated cells.

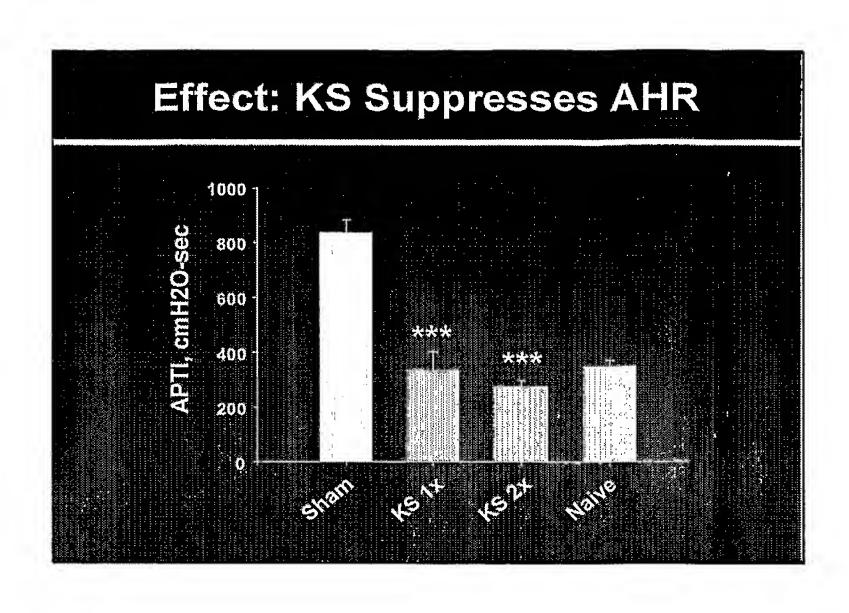


FIGURE 23

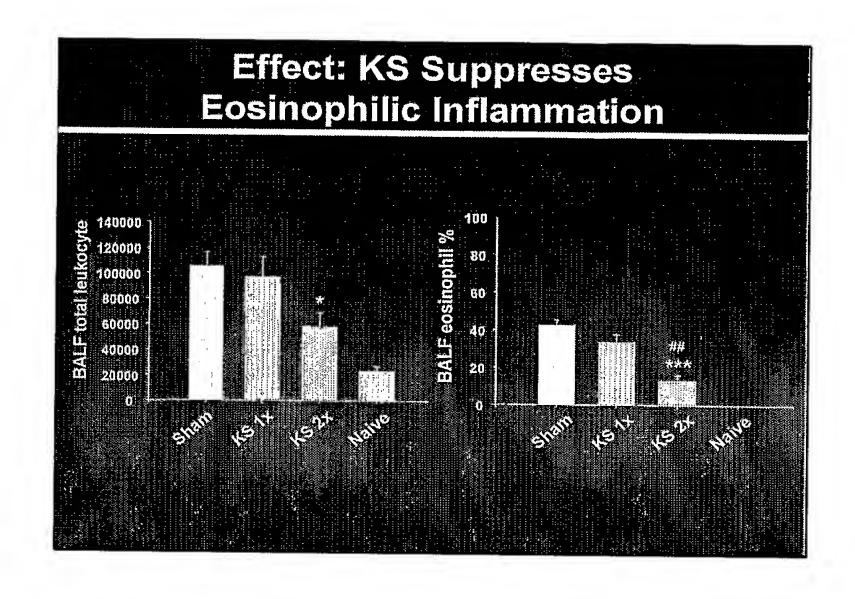


FIGURE 24

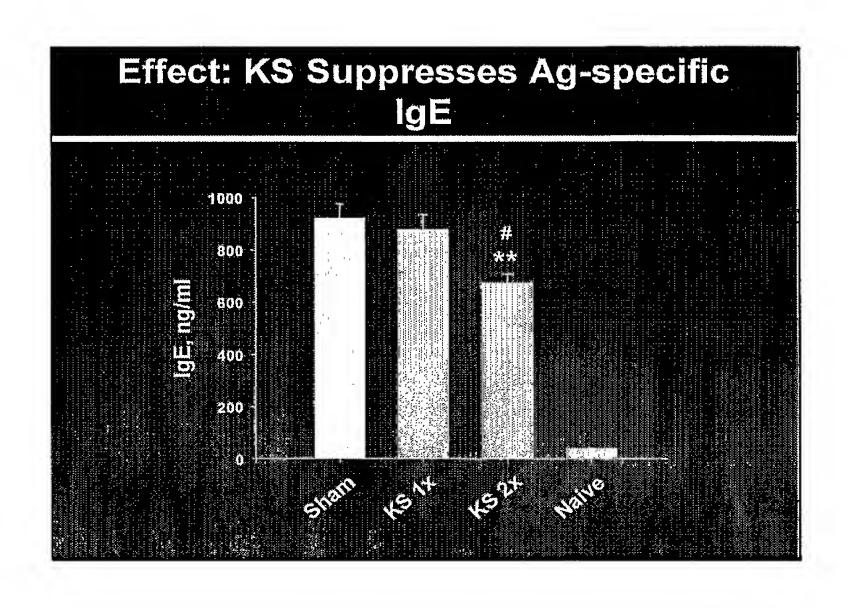


FIGURE 25

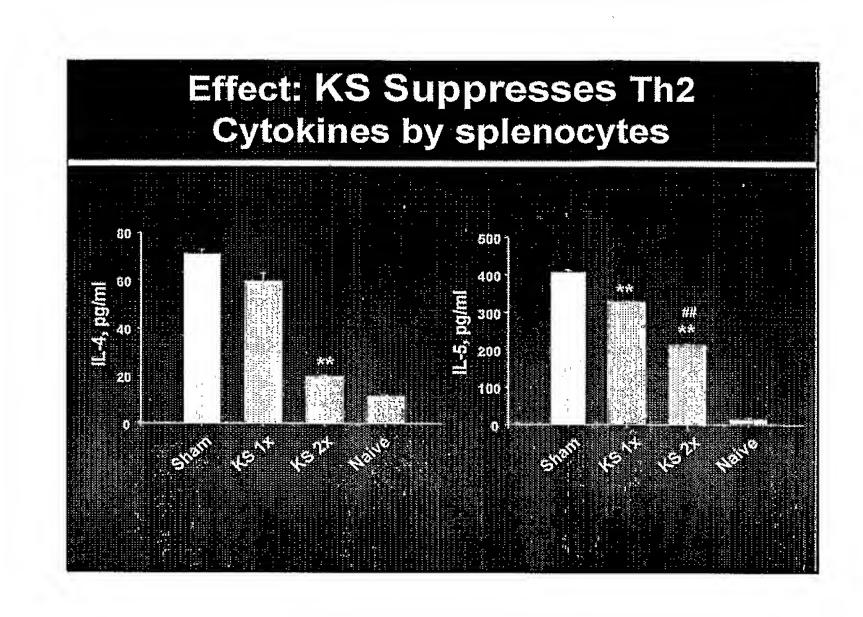


FIGURE 26

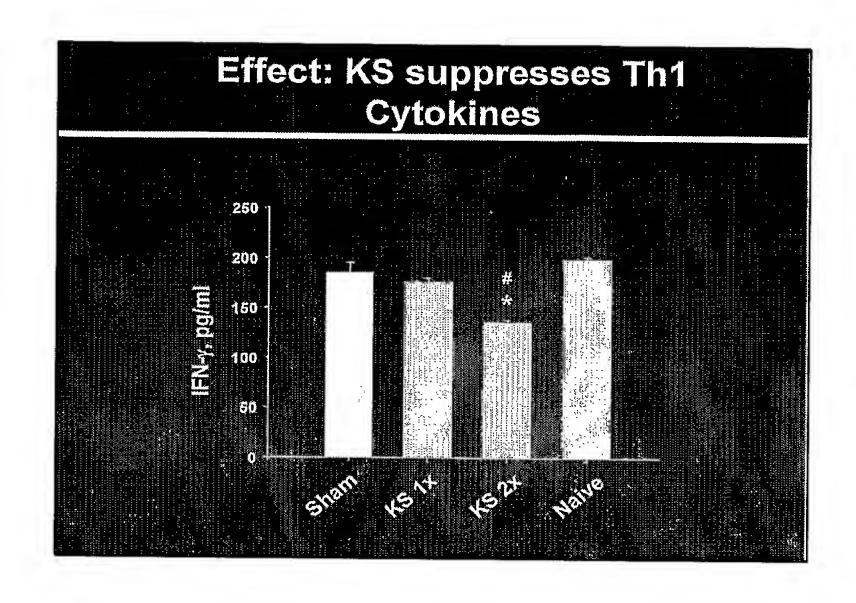


FIGURE 27

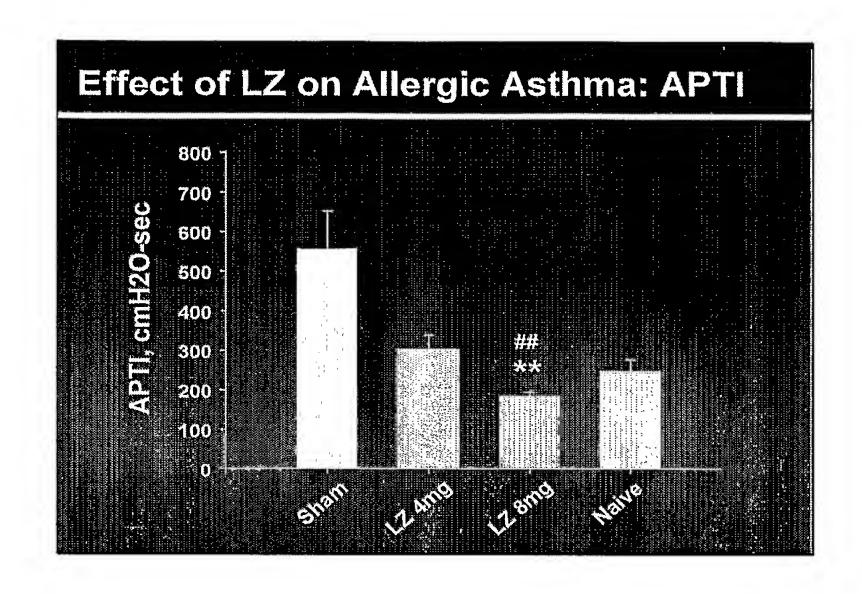


FIGURE 28

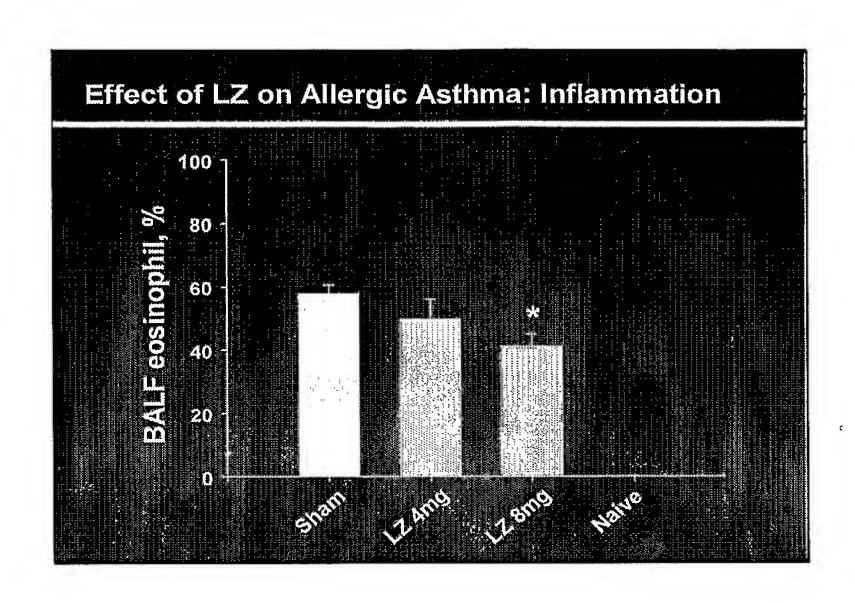


FIGURE 29

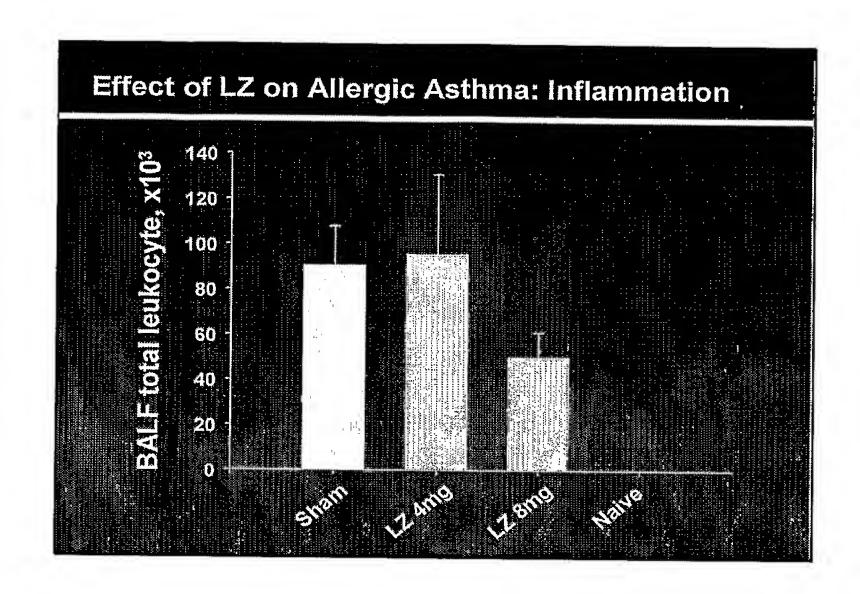


FIGURE 30

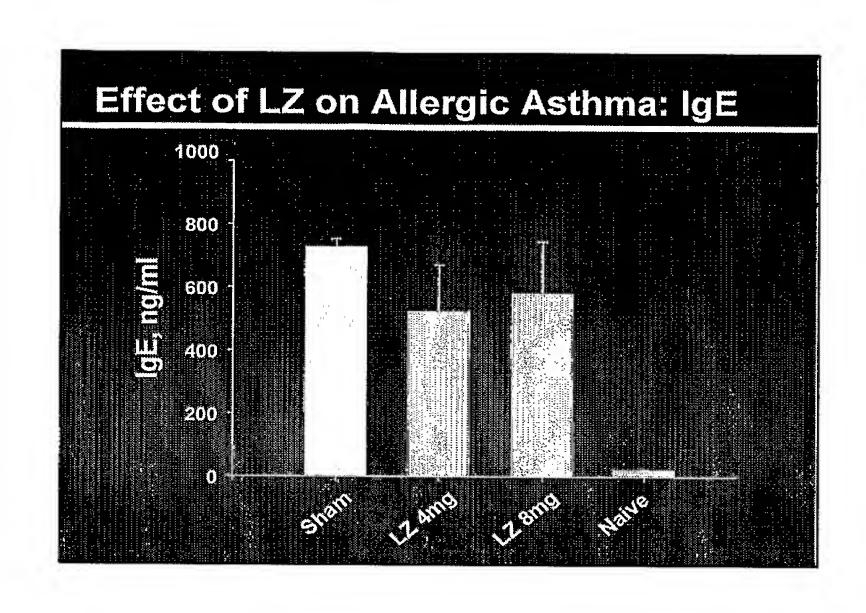


FIGURE 31

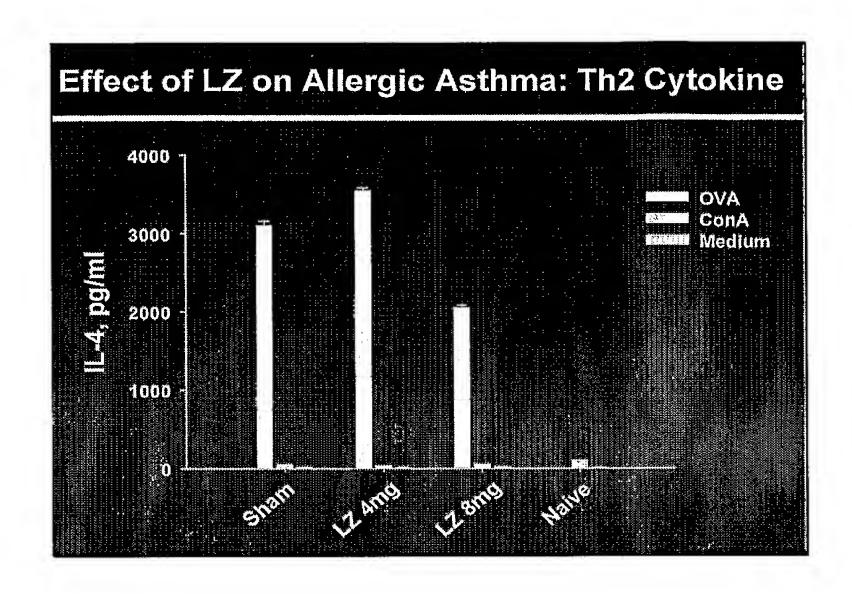


FIGURE 32

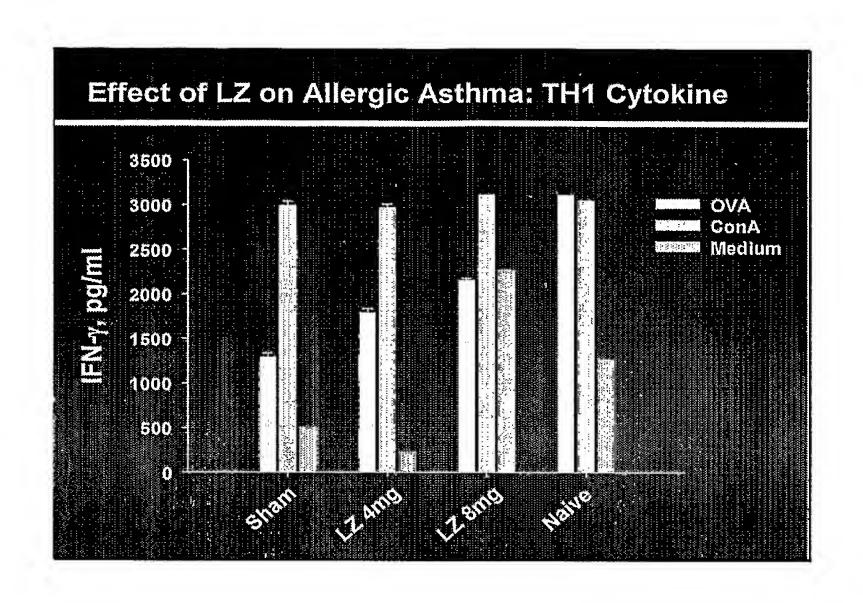


FIGURE 33

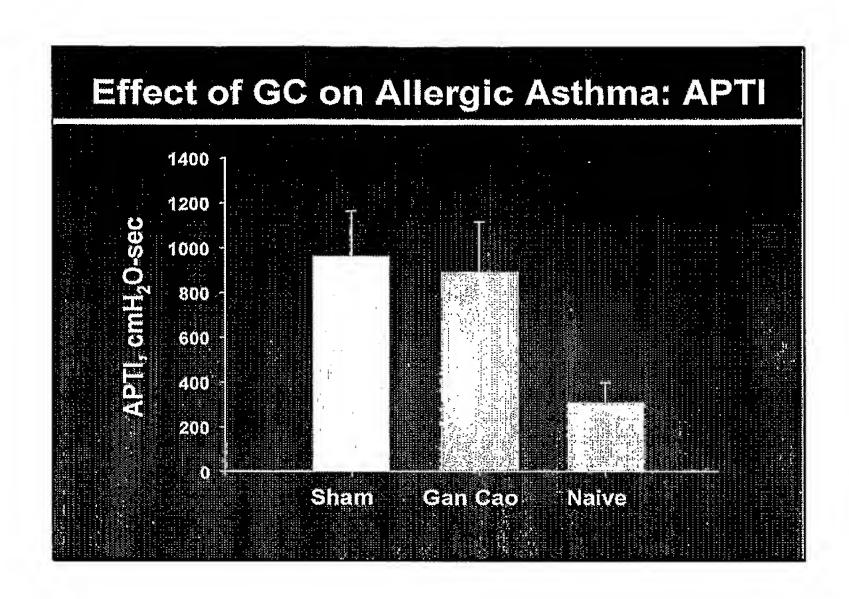


FIGURE 34

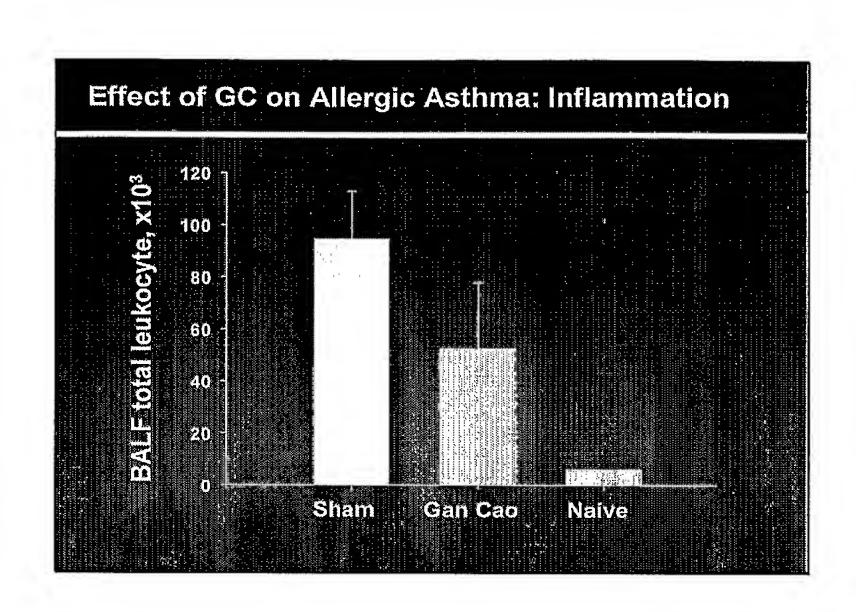


FIGURE 35

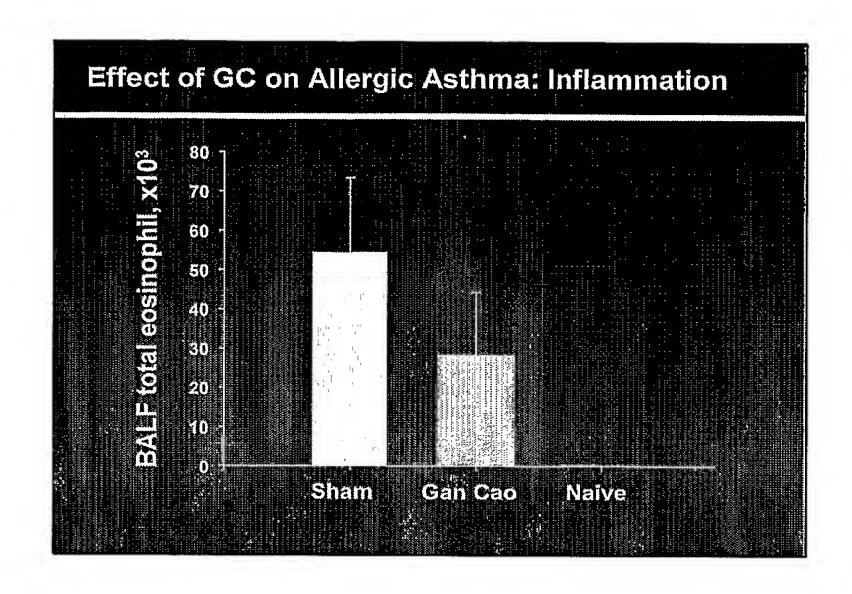


FIGURE 36

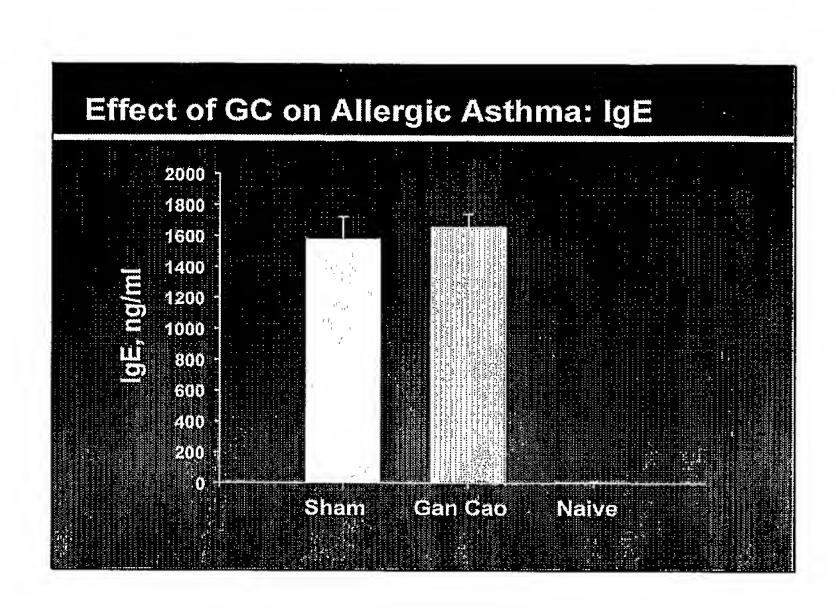


FIGURE 37

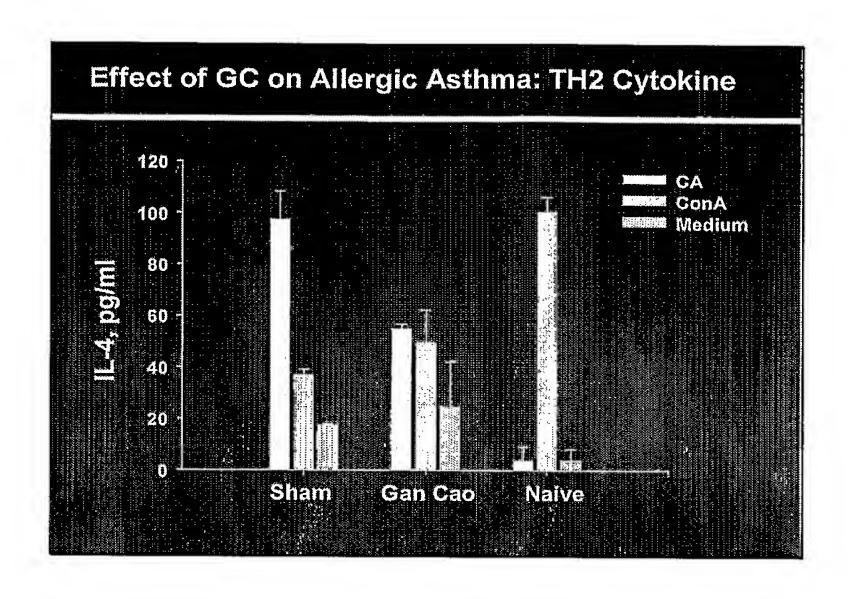


FIGURE 38

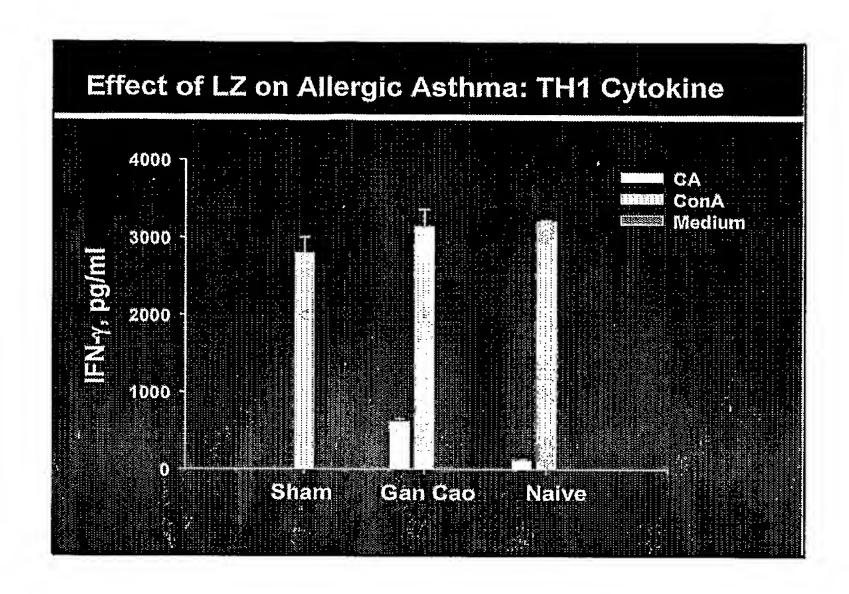


FIGURE 39

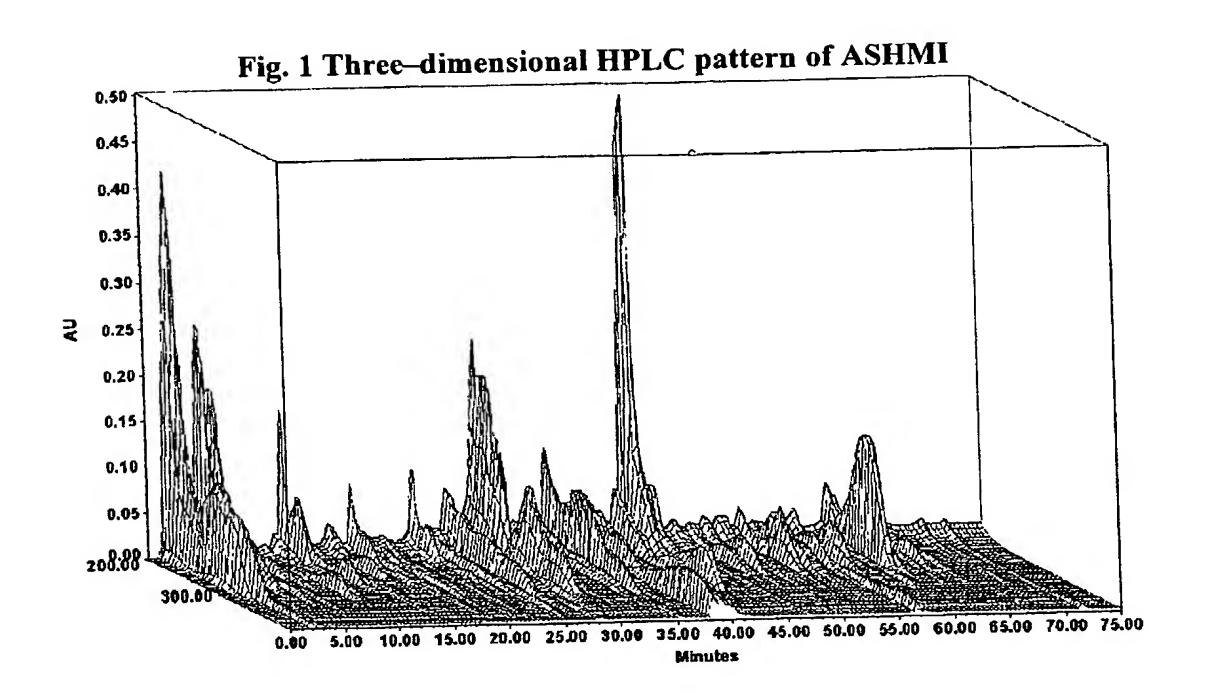


FIGURE 1